ISSN (print) 0093-4666 © 2015. Mycotaxon, Ltd. ISSN (online) 2154-8889

MYCOTAXON

http://dx.doi.org/10.5248/130.857

Volume 130, pp. 857–865 *July–September 2015*

Volvariella rava sp. nov. from southern China

Jiang Xu[1, 2], Tai-Hui Li[2*], Ya-Heng Shen[2], & Ming Zhang[1, 2]

[1] *School of Bioscience & Bioengineering, South China University of Technology, Guangzhou 510006, China,*

[2] *State Key Laboratory of Applied Microbiology Southern China, Guangdong Provincial Key Laboratory of Microbial Culture Collection and Application, Guangdong Institute of Microbiology, Guangzhou 510070, China*

*Correspondence to: *mycolab@263.net*

Abstract—*Volvariella rava*, a new grey species collected from Guangzhou City in southern China, is described, illustrated, and compared with closely related taxa of *Volvariella*. The new species is characterized by a medium-sized basidiome and greyish brown appressed-fibrillose pileus; it closely resembles *V. volvacea* but differs by its smaller spores. Molecular analysis based on nrITS sequences confirmed that *V. rava* is distinct from other morphologically similar *Volvariella* species.

Key words—*Agaricales*, *Basidiomycota*, fungal diversity, phylogeny, taxonomy

Introduction

Volvariella Speg. (traditionally referred to *Pluteaceae,* but see Justo et al. 2011) is characterized by a small to large agaricoid basidiome having a slender to fleshy stipe with a volva but no ring, crowded and free (often remote) lamellae, and pink basidiospores in mass (Orton 1986). The genus has been reported from tropical, subtropical, and temperate regions of both the eastern and the western hemispheres (Shaffer 1957). A review of the bibliographical and herbarium records (Mycological Herbarium of Microbiology Institute the Chinese Academy of Science (HMAS), Herbarium of Cryptogams, Kunming Institute of Botany Academy of Science (HKAS), Mycological Herbarium of Mycological Institute of Jilin Agriculture University (HMJAU), and Mycological Herbarium of Microbiology Institute Guangdong Academy of Science (GDGM)) revealed that a total of 22 *Volvariella* species have been described and reported from China (Teng 1963, Tai 1979, He & Feng 1987, Bi et al. 1994, Ying & Zang 1994, Shao & Xiang 1997, Mao 2000, Li et al. 2009, Bao

& Wang 2013). A grey species of *Volvariella* discovered from Tianlu Lake Forest Park of Guangzhou City, southern China, is described here as a new species based on morphological and molecular characters.

Table 1. Sequences of *Volvariella* (*V.*) and outgroup species used in the phylogenetic analyses.

Species	Voucher No.	GenBenk No.
Volvariella bombycina	AJ244	HM562212.1
	SP393635	EU920673.1
V. caesiotincta	MA54717	HM562211.1
V. dunensis	SCM3513	JF415140.1
V. hypopithys	AV137	HM246492.1
V. nivea	GDGM25489	FJ749127.1
V. nullicystidiata	SP393639	EU920671.1
V. pusilla	AJ51(LOU)	JF415137.1
	AV139	HM246494.1
V. rava	**GDGM41955**	**KP784686**
V. sathei	AMH 9436	JN792550.1
V. cf. *sathei*	Hama193	KF926663.1
	Hama390	KF926666.1
	Hama194	KF926664.1
	Hama204	KF926665.1
V. strangulata	AV141	HM246493.1
V. surrecta	AJ55	HM562213.1
V. taylorii	AJ54	HM562210.1
V. terrea	LUG11010	JF415141.1
V. volvacea	AV143	HM246500.1
	ATCC MYA-4696*	HQ999973.1
Volvariella sp.	LOU18924	JF415139.1
Volvopluteus earlei (as *V. acystidiata*)	HG1973	HM246499.1
Volvopluteus earlei (as *V. media*)	HG2001	HM246498.1
Volvopluteus earlei (as *V. cookei*)	AV133	HM246496.1
Volvopluteus earlei (as *V. earlei*)	AV134	HM246497.1
Schizophyllum commune	IFM 56967*	AB566277.1

* Strain no.

Material & methods

The macroscopic descriptions were based on the fresh basidiomata (Vellinga 1988). The microscopical characters were observed using an Olympus DP25 microscope. Thin handmade sections were made from dried specimens and revived in 5% KOH.

The pileipellis and stipitipellis were examined in KOH and basidiospores, basidia and cystidia were observed in 1% Congo red. Basidiospore dimensions are based on the random measurements of 30 basidiospores. The abbreviation [n/m/p] represents measurements made on n basidiospores in m basidiomata from p collections. Basidiospores dimensions follow the form (a–)b–c(–d) where b–c represents 95% of the measured values and extreme values shown in parentheses. The following abbreviations are used in the descriptions: avl for average length, avw for average width, Q for quotient of length/width, avQ for average quotient. Colour designations follow Kornerup & Wanscher (1978). The type specimen is deposited at the Herbarium of Guangdong Institute of Microbiology, Guangzhou, China (GDGM).

Genomic DNA of the new species was extracted from herbarium materials using the Sangon Fungus Genomic DNA Extraction kit (Sangon Biotech Co., Ltd., Shanghai, China) according to the manufacturer's instructions. The ITS region was amplified by PCR with universal primers ITS4 and ITS5 (White et al. 1990). The amplified strands were sequenced with an ABI 3730 DNA analyzer (Invitrogen Life Technologies Co., Ltd, Shanghai). The newly generated sequences were submitted to GenBank. For phylogenetic analysis, additional related sequences of *Volvariella* obtained from NCBI (Table 1) were edited and aligned by Clustal W (Thompson et al. 1994) and manually checked and adjusted. The outgroup comprised *Schizophyllum commune* Fr. and *Volvopluteus earlei* (Murrill) Vizzini et al. A phylogenetic tree was generated with MEGA v.6.0 (Tamura et al. 2013). All characters were treated as unordered and equal weight. Gaps were treated as missing data. Bootstrap values were calculated for 1000 replicates.

Taxonomy

Volvariella rava T.H. Li & Jiang Xu, **sp. nov.** Plates 1, 2

MycoBank MB 811615

Differs from *Volvariella volvacea* by its smaller basidiospores, glossy pileus with appressed fibrils, and irregularly lobed volva.

Type: China, Guangdong Province, Guangzhou City, Tianlu Lake Forest Park, 23°13.38′N 113°25.05′E, alt. 50–100 m asl, 14.09.2012, Zhang M, Zhou SH (**Holotype**, GDGM41955; GenBank KP784686).

Etymology: The epithet '*rava*' refers to the tawny grey to grayish brown colour of the pileus.

Basidiomata medium. Pileus 25–60 mm diam., ovoid when young, becoming subconical with an obtuse apex, convex at maturity; surface tawny grey to greyish brown (5E3-5F3), darker at the disc, subviscid to viscid when wet, nearly glabrous to innately appressed-fibrillose; margin estriate, often radially rimulose on drying, eroded. Context white, unchanging on bruising, 5–6 mm thick near stipe. Lamellae free, remote, white when young, becoming sordid and pinkish, ≤6 mm wide, densely crowded, with lamellulae of different lengths. Stipe 70–80 × 5–10 mm, cylindrical, compressed, slightly attenuated

towards apex, white, glabrous, solid. Volva free, concolorous with the pileus above, whitish (4A2) below, irregularly lobed, relatively thick, ≤12 mm high and 15 mm wide. Odour and taste mild.

Basidiospores (60/2/2) (4–)5–6.5 × 4–5 µm, (avl × avw = 5.47 × 4.28), Q = 1.1–1.5, avQ = 1.28, ovoid to ellipsoid, smooth, stramineous to salmon-coloured, pinkish at thickened wall. Basidia 24–30 × 7–10 µm, cylindric-clavate to clavate, 4-spored, rarely 2-spored; sterigmata short (≤2 µm), slender. Lamellar edge heteromorphous. Cheilocystidia abundant, 55–80 × 15–25 µm, fusoid to ventricose, or subclavate with blunt end, thin-walled, easily collapsing. Pleurocystidia scattered, 35–71 × 11–30 µm, cylindric-clavate, mucronate with a broadly rounded apex, thin-walled, similar to cheilocystidia. Lamellar trama convergent, composed of hyaline hyphae, 4–7 µm diam., thin-walled. Pileipellis a regular cutis consisting of procumbent hyphae, 12–23 µm diam., gelatinous, thin-walled; terminal elements blunt or slightly acute, 54–86 × 10–18 µm, hyaline. Stipitipellis hyphae cylindric, rarely septate, 7–25 µm diam., with rounded or slightly acute apex. Volva made up of filamentous hyphae, with inflated cells (32–50 × 10–15 µm) at outer and inner surfaces, often containing brownish vacuolar pigments. Caulocystidia absent. Clamp connections absent in all hyphae.

Habitat & Distribution—Solitary on ground of bamboo grove in a subtropical evergreen broad-leaved forest. Known only from the type locality.

Additional specimen examined: **China**, **Guangdong Province**, Guangzhou City, Tianlu Lake Forest Park, 12.05.2011, Zhang M, Xu J (GDGM41548).

Phylogenetic analyses

The 788 bp nrITS sequence from the new species (GDGM41955) differs from all other known *Volvariella* sequences deposited in the NCBI-GenBank database (http://www.ncbi.nlm.nih.gov/).

An initial BLASTn search comparing the nrITS sequence from *V. rava* with the NCBI nrITS sequence database showed maximum 98% similarity with *V. nivea* T.H. Li & Xiang L. Chen (FJ749127) followed by 96% similarity with *V. strangulata* (Romagn.) Vizzini & Contu (HM246493) and *V. dunensis* (Vila et al.) Justo & M.L. Castro (JF415140), 90% similarity with *V. surrecta* (Knapp) Singer (HM562213), and 84% similarity with *V. caesiotincta* P.D. Orton (HM562211).

Twenty-six additional sequences obtained from NCBI, representing 15 *Volvariella* spp. and an outgroup of *Volvopluteus earlei* (deposited as *Volvariella*) and *Schizophyllum commune*, were included in the analyses. In the phylogenetic tree (Plate 3), all *Volvariella* species were grouped in two main clades I and II. *Volvariella rava* and *V. nivea* clustered in subclade Ib with

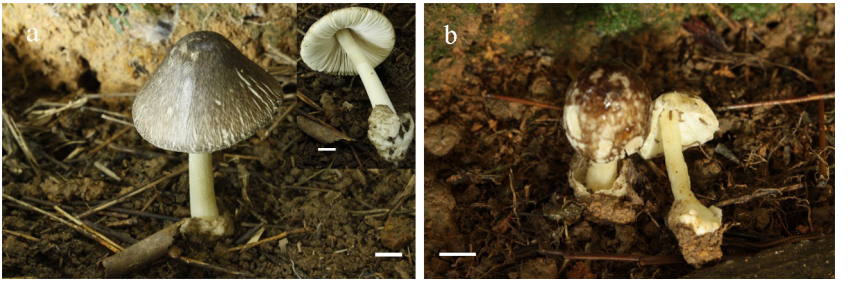

Plate 1. *Volvariella rava*: basidiomata in the natural habitat. a. Surface and gill views (holotype, GDGM41955); b. young basidiomata (GDGM41548). Scale bars = 1cm.

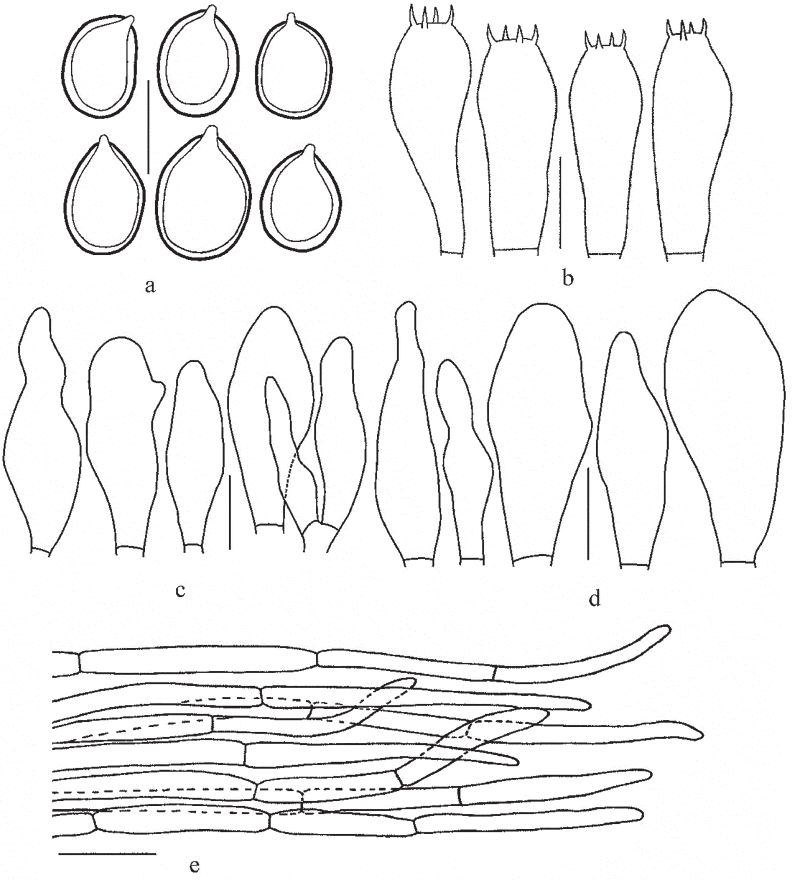

Plate 2. *Volvariella rava* (holotype, GDGM41955). a. Basidiospores; b. basidia; c. cheilocystidia; d. pleurocystidia; e. pileipellis elements. Scale bars: a = 5 μm; b = 10 μm; c, d = 20 μm; e = 40 μm.

84% boot strap support, but the different branch lengths imply considerable sequence divergence between these two species.

In this study, a high degree of variability (including considerable misalignment and several indels) among the *Volvariella* species was notable throughout the ITS 1 and ITS 2 regions, although the 5.8S region had few changes and remained conserved, as indicated by Li et al. (2009), Vizzini et al. (2011), and Senthilarasu et al. (2012) for *V. nivea* and *V. sathei* Senthil. et al.

Discussion

Volvariella rava can be placed in stirps *Volvacea* sect. *Volvariella* based on its medium-sized pileus with appressed fibrils and small (≤12 µm long) basidiospores (Singer 1986, Justo et al. 2011). *Volvariella rava* closely resembles other species in stirps *Volvacea: V. volvacea* (Bull.) Singer, *V. bakeri* (Murrill) Shaffer, *V. esculenta* (Massee) Singer, and *V. diplasia* (Berk. & Broome) Singer. However, *V. volvacea* is distinguished from *V. rava* by its white to dark erect pileus fibrils and much larger basidiospores (7–10 × 4.5–7 µm; Shaffer 1957, Pegler 1977, 1986, Priest & Conde 2006). *Volvariella bakeri* has a dark-fuliginous pileus with concolorous volva and larger basidiospores (6.9–9.3 × 4.6–6.9 µm; Shaffer 1957). *Volvariella esculenta* differs from *V. rava* in having a glabrous grayish violet pileus with striate margin and elongate basidiospores (6–7 × 4–5 µm; Massee 1908, Singer 1949). *Volvariella diplasia* has a white to golden yellow silky-floccose pileus and larger basidiospores (7.5–10.5 × 4.7–6.5 µm; Singer 1949, Pegler 1986).

Volvariella rava somewhat resembles *V. apalotricha* (Berk. & Broome) Pegler, *V. pseudovolvacea* (Berk. & Broome) Singer, and *V. terastia* (Berk. & Broome) Singer in its medium-sized basdiomata, grayish to dark pileus, and ≤7 µm long basidiospores. However, *V. apalotricha* has an ash-grey to greyish brown pileus with blackish radial fibrils, a small white membranous volva, narrower basidiospores (3.5–4.5 µm diam.), and ventricoso-clavate cheilocystidia and pleurocystidia (Pegler 1986, Pradeep et al. 1998, Priest & Conde 2006). *Volvariella pseudovolvacea* has dense black fibrillose striae on the pileus, slightly smaller basidiospores (4.2–5.2 × 2.5–3.5 µm), and fusoid ventricose to lageniform cheilocystidia (Pegler 1986, Bi et al. 1994, Pradeep et al. 1998). *Volvariella terastia* differs from *V. rava* in its larger basidiome and pileus (60–110 mm diam.) covered with dark brown fibrils, thicker stipe (≤20 mm diam., ≤30 mm thick at the base), larger volva (≤80 mm high), and smaller cheilocystidia (32–36 × 10–13 µm; Pegler 1986).

Volvariella aethiops A. Favre & Vialard, *V. caesiotincta*, *V. lepiotospora* Singer, and *V. taylorii* (Berk. & Broome) Singer are also somewhat similar to *V. rava* in their greyish brown or darker pileus colour. However, *V. aethiops* has

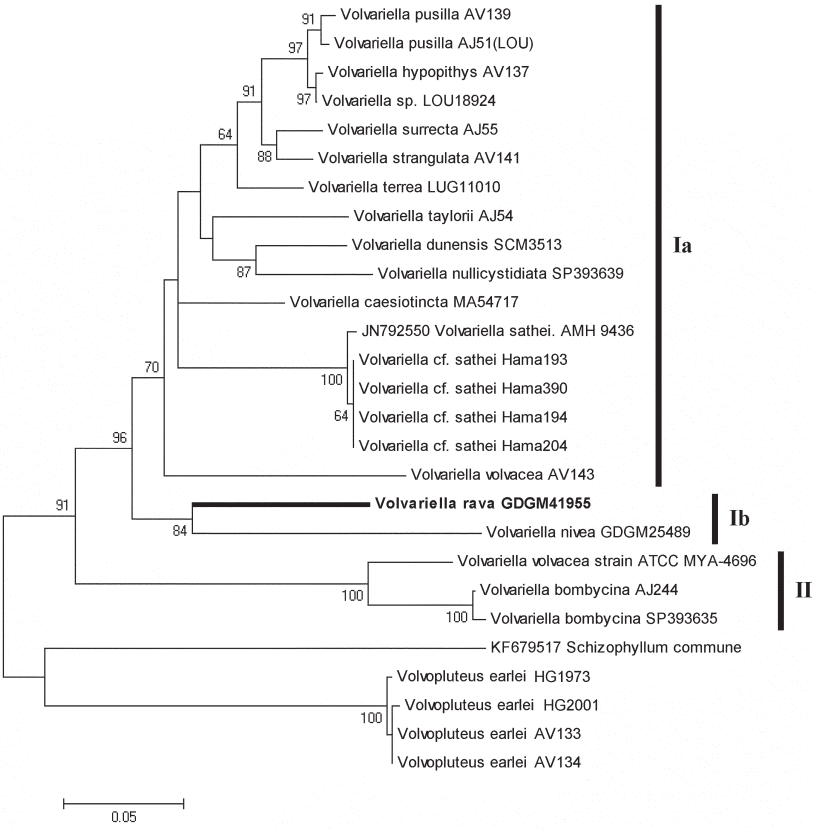

Plate 3. Phylogenetic tree of *Volvariella* sequences generated from ITS dataset using NJ method. Bootstrap values ≥50% are shown on the branches. Scale bar indicates nucleotide substitutions per site.

a distinctly shaggy fibrillose pileus and longer basidiospores (6.2–8.6 × 4–4.7 μm; Favre & Vialard 2007, Kibby 2011). *Volvariella caesiotincta* is lignicolous and has greyish white to greyish brown to pale bluish pileus, a membranous and often 3-lobed volva, and narrow basidiospores (5.5–7.5 × 3.3–4 μm; Orton 1974, 1986). *Volvariella lepiotospora* differs from *V. rava* in its smaller (32 mm diam.) pileus covered by brownish black to black fibrils and narrower basidiospores (4.7–6(–7) × 3–3.7 μm; avQ = 1.67; Singer 1955, Shaffer 1957). *Volvariella taylorii* is separated by its silky fibrillose pileus, smaller stipe (35–55 × 2–4 mm), and smaller lageniform pleurocystidia (32–48 × 8–12 μm), and cheilocystidia (25–45 × 9–13 μm; Pegler 1983, Pradeep et al. 1998).

Volvariella nivea was described from the Baiyun Mountain Forest Park (very close to Tianlu Lake Forest Park) growing on the ground of a bamboo grove. It thus has a similar habitat to *V. rava* and shares similar spore dimensions (6–7 × 4.5–5.5 µm), but clearly differs in its snow-white basidiomata with the distinctly free-ended fibrils on the pileus (Li et al. 2009).

Acknowledgments

We would like to thank Dr. Alfredo Justo (Universidad Nacional Autónoma de México, Mexico) and Dr. Gunasekaran Senthilarasu (SRM Research Institute, SRM University, Tamil Nadu, India) for their pre-submission reviews. This study was supported by the National Natural Science Foundation of China (Nos. 31093440, 31170026, 31370072) and the early-stage basic research key projects of National Basic Research Program of China (2014CB460613).

Literature cited

Bao T, Wang JR, Cui BK, Liu Y. 2013. Diversity of macrofungi in Shandong Province, China. Mycosystema 32(4): 643–670.

Bi ZS, Zheng GY, Li TH. 1994. The macrofungus flora of China's Guangdong Province. Guangdong science and technology press. 879 p.

Favre A, Vialard J. 2007. *Volvariella aethiops* nov. sp. Bulletin Mycologique et Botanique Dauphiné-Savoie 47(184): 19–23.

He SC, Feng L. 1987. A new species of the genus *Volvariella* from Guizhou Province of China. Acta Mycologia Sinica 6(3): 150–152.

Justo A, Vizzini A, Minnis AM, Menolli N Jr., Capelari M, Rodriguez O, Malysheva E, Contu M, Ghignone S, Hibbett DS. 2011. Phylogeny of the *Pluteaceae* (*Agaricales*, *Basidiomycota*): taxonomy and character evolution. Fungal Biology 115: 1–20. http://dx.doi.org/10.1016/j.funbio.2010.09.012

Kibby G. 2011. *Volvariella aethiops*, the first British collection. Fungal Portraits 12(3): 75–76.

Kornerup A, Wanscher HH. 1978. Methuen handbook of colour. Eyre Methuen, London. 243 p.

Li TH, Chen XL, Shen YH, Li T. 2009. A white species of *Volvariella* (*Basidiomycota*, *Agaricales*) from southern China. Mycotaxon 109: 255–261. http://dx.doi.org/10.5248/109.255

Mao XL. 2000. The macrofungi in China. Henan Science and Technology Press. 719 p.

Massee GE. 1908. Fungi exotici, VIII. Bulletin of Miscellaneous Information, Royal Botanical Gardens, Kew 1908: 216–219

Orton PD. 1974. The European species of *Volvariella*. Bulletin de la Société Linnéenne de Lyon, num. special 43: 313–326.

Orton PD. 1986. British fungus flora. *Agarics* and *Boleti*. 4: *Pluteaceae*: *Pluteus* and *Volvariella*. Edinburgh, Royal Botanic Garden. 98 p.

Pegler DN. 1977. A preliminary agaric flora of East Africa. Kew Bulletin Additional Series 6. 615 p.

Pegler DN. 1983. Agaric flora of the Lesser Antilles. Kew Bulletin Additional Series 9. 668 p.

Pegler DN. 1986. Agaric flora of Sri Lanka. Kew Bulletin Additional Series 12. 519 p.

Pradeep CK, Vrinda KB, Mathew S, Abraham TK. 1998. The genus *Volvariella* in Kerala State, India. Mushroom Research 7(2): 53–62.

Priest M, Conde B. 2006. The occurrence of *Volvariella volvacea* (Bull. : Fr) Singer in the Northern Territory, Australia. Australasian Mycologist 25(2): 65–67.

Senthilarasu G, Sharma R, Singh SK. 2012. A new species of *Volvariella* from India. Mycotaxon 119: 467–476. http://dx.doi.org/10.5248/119.467

Shaffer RL. 1957. *Volvariella* in North America. Mycologia 49(4): 545–579. http://dx.doi.org/10.2307/3756159

Shao LP, Xiang CT. 1997. Forest mushrooms of China. Northeast Forestry University Press, Harbin. 652 p.

Singer R. 1951 ["1949"]. The *Agaricales* (mushrooms) in modern taxonomy. Lilloa 22. 832 p.

Singer R. 1955. New and interesting species of basidiomycetes. IV. Mycologia 47(5):763–777. http://dx.doi.org/10.2307/3755585

Singer R. 1986. The *Agaricales* in modern taxonomy (4th edition). Koenigstein, Koeltz Scientific. 455–457.

Tai FL. 1979. Sylloge Fungorum Sinicorum. Science Press: Beijing. 811 p.

Tamura K, Stecher G, Peterson D, Filipski A, Kumar S. 2013. MEGA6: Molecular Evolutionary Genetics Analysis Version 6.0. Molecular Biology and Evolution 30(12): 2725–2729. http://dx.doi.org/10.1093/molbev/mst197

Teng SC. 1963. Fungi of China. Science Press: Beijing. 808 p.

Thompson JD, Higgins DG, Gibson TJ. 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Research 22: 4673–4680. http://dx.doi.org/10.1093/nar/22.22.4673

Vellinga EC. 1988. Glossary. 54–64, in: C Bas et al. (eds). Flora Agaricina Neerlandica 1. A.A. Balkema, Rotterdam.

Vizzini A, Contu M, Justo A. 2011. Additional records of *Volvariella dunensis* (*Basidiomycota, Agaricales*): morphological and molecular characterization. Mycotaxon 117: 37–43. http://dx.doi.org/10.5248/117.37

White TJ, Bruns T, Lee S, Taylor J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenies. 315–322, in: MA Innis et al. (eds). PCR protocols: a guide to methods and applications. Academic, San Diego.

Ying JZ, Zang M. 1994. Economic macrofungi from southwestern China. Science Press. 399 p.